REMARKS

Applicant acknowledges that Claims 5-6 and 12-13 are objected to as depending upon a rejected base claim but that Claims 5-6 and 12-13 are free of the prior art of record.

Claim Rejection Under 35 U.S.C. §102

Claims 1-4, 7-11, and 14-20 are rejected under 35 U.S.C. §102(b) as anticipated by or, in the alternative, under 35 U.S.C. §103(a) as obvious over Roy.

In the Office Action, the Examiner stated that washing steps are encompassed within the scope of the claims and that in the method disclosed by Roy, some residual amount of potassium dichromate remains in contact with the specimen. Further, the Examiner stated that the 7.8 pH Holmes' buffer disclosed by Roy is a source of hydrogen ions.

As previously noted, Applicant's claimed invention is directed to a method of staining wherein a biological specimen is treated by a process that includes treatment with a corrosive reagent. In the process, an oxidizer, e.g., chromate ions, and an acid source of hydrogen ions are dispensed onto the biological specimen.

While the claims do not rule out washing steps, they do, however, specify that the "the oxidizer combines with the hydrogen ions and the combination of oxidizer and hydrogen ions contacts the biological specimen, thereby treating the biological specimen with the corrosive reagent." (Claim 1.) Thus any washing steps that might be carried out according to Applicant's claimed method, are not such that they remove the oxidizer, since, as noted above, the oxidizer is present in order for it to combine with the hydrogen ions.

In contrast to Applicant's claimed invention, as further discussed below, Roy does not disclose or suggest: (i) the combination of oxidizer and hydrogen ions; or (ii) dispensing an acid source of hydrogen ions.

Removal of the Potassium Dichromate

In the method described by Roy, the sections are exposed to 10% potassium dichromate (Step 2); and subsequently to hexamine solution containing pH 7.8 Holmes' boric acid-Borax buffer (Step 7).

Specifically, Roy discloses at Step 2 the exposure to 10% potassium dichromate for 1 hour at room temperature. However, this step is immediately followed by a step of washing in

tap water for 5 minutes (Step 3). This step is intended to, and invariably will, remove at least the bulk of the potassium dichromate from the sample.

In the following step, Step 4, the slides are "flood"ed with 3% sodium metabisulfite for 2 minutes. As is known in the art, sodium metabisulfite is a reducing agent; accordingly, treatment with sodium metabisulfite reduces any potassium dichromate remaining on the slides. That is to say, the sodium metabisulfite neutralizes any residual oxidizing activity of any remaining potassium dichromate. Thus, whatever trace amount of potassium dichromate oxidizer that may have been left on the slide following the tap water washing of Step 3 are inactivated or rendered incapable of oxidizing by the sodium metabisulfite during Step 4.

Finally, the reducing step is followed by several further washing steps. At Step 5, the slide is washed in running water for 5 minutes and at Step 6, the slides are washed well in four changes of distilled water.

In summary, as a result of the washing, neutralization and further washing steps disclosed by Roy, potassium dichromate is effectively removed from the sections and no potassium dichromate oxidizer remains on the slide by the time that, in Step 7, the sample comes into contact with the pH 7.8 Holmes' boric acid-Borax buffer. Accordingly, even if the pH 7.8 Holmes' boric acid-Borax buffer were to be considered an "acid source of hydrogen ions" (separately discussed below), the method of Roy cannot and does not disclose or suggests that "the oxidizer combines with hydrogen ions and the combination of oxidizer and hydrogen ions contacts the biological specimen, thereby treating the biological specimen" (Claim 1).

Boric Acid Buffer

At Step 7 of the method disclosed by Roy, the sample is exposed to hexamine solution, which is made by adding 8 ml of Holmes' buffer, having a pH of 7.8, to 30 ml stock silver solution. The reference teaches that Holmes' buffer is prepared by adding 8 ml M/5 boric acid to 2 ml M/20 borax.

As known in the art, a buffer is a solution which is capable of withstanding changes to pH when hydrogen ions (acids) or hydroxyl ions (alkalis) are added to the solution. As the name suggests, a buffer is used to maintain a constant pH. Buffers are made from solutions comprising: (i) a weak acid or weak base, and (ii) the conjugate base or acid. Copies of an entry in the Oxford Dictionary of Biochemistry and Molecular Biology, as well as relevant pages in Stryer's Biochemistry, which explain the properties of buffers, are enclosed.

In the case of Holmes' buffer which is a boric acid - borate buffer, Roy clearly states that the pH of this buffer is 7.8. As is well known, pH values below 7 are acidic, while those above 7 are alkaline or basic. Accordingly, the hexamine solution of Roy cannot be considered "an acid source of hydrogen ions". Rather, the buffer comprising boric acid and borax disclosed in Roy is clearly alkaline.

Thus, contrary to the Examiner's statements, Roy does not teach a method of staining a biological specimen with a histological stain, comprising the step of dispensing onto the biological specimen "an acid source of hydrogen ions". Rather, the cited reference teaches the skilled artisan to do the opposite from what is claimed by Applicant, i.e., to expose the sample to a basic solution.

Furthermore, Roy neither discloses nor suggests Applicant's Claims 18-20, which include the step of dispensing from independent liquid dispensers, onto the specimen, precursors of the corrosive reagent, wherein the liquid dispensers include at least one part fabricated from a material incompatible with the corrosive reagent and wherein the precursors are less corrosive to the material than is the corrosive reagent, whereby the precursors combine *in situ* to form the corrosive reagent, thereby treating the specimen.

Therefore, Applicant respectfully submits that Claims 1-4, 7-11 and 14-20 are neither anticipated by nor obvious over Roy.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned.

Respectfully submitted,

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Concord, MA 01742-9133
Dated: November 25, 2003

OXFORD DICTIONARY OF

BIOCHEMISTRY AND MOLECULAR BIOLOGY

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protein 3-α-galactosyltransferase; *other name*: [histo-blood group] B transferase; an enzyme that catalyses the reaction:

UDPgalactose + glycoprotein α-L-fucosyl-(1,2)-D-galactose = UDP + glycoprotein α-D-galactosyl-(1,3)-[α-L-fucosyl-(1,2)]-L-galactose,

thus adding galactose to the H antigen of the **ABH antigen** system, leading to formation of the B antigen. The protein is a product of one allele of the *ABO* gene. It has virtual identity of sequence with that catalysing **A-transferase** activity, differing only at residues 176 (A has Arg, B has Gly), 235 (A Gly, B Ser), 266 (A Leu, B Met), and 268 (A Gly, B Ala). Database code BGAT_HUMAN, 354 amino acids (40.89 kDa).

Btu *abbr. for* British thermal unit.

Bu *symbol for* the butyl group.

BU *(sometimes) abbr. for* bromouracil (BrUra is recommended).

bubble column a column-shaped bioreactor in which the reaction medium is kept mixed and aerated by the introduction of air at the bottom.

Büchner, Eduard (1860–1917), German chemist and biochemist renowned for his seminal discovery that alcoholic fermentation could be initiated with a cell-free press juice from brewers' yeast, the active principle of which he considered to be a protein denoted zymase; Nobel Laureate in Chemistry (1907) 'for his biochemical researches and his discovery of cell-free fermentation'.

Büchner funnel a cylindrical funnel for filtration, usually of porcelain or plastic, that includes a perforated plate on which a filter paper is placed. It is generally used with a vacuum. *See also* **Hartley filter funnel**.

bud 1 a small lateral or terminal protuberance on the stem of a plant that contains undeveloped foliage or floral leaves. 2 a budlike protuberance on the surface of a yeast cell or other simple organism. 3 to form a bud. 4 to reproduce asexually, as in yeasts, by the process of **budding**.

Buddha suite of computer programs for structure refinement and energy minimization.

budding 1 the production of a bud or buds. 2 a form of asexual reproduction, occurring in certain bacteria and fungi (e.g. yeasts) and some primitive animals in which an individual arises from a daughter cell formed by pinching off a part of the parent cell. The budlike outgrowths so formed may sometimes remain attached to the parent cell.

BUdR *or* BUDR *(sometimes) abbr. for* bromodeoxyuridine (the symbol BrdUrd is preferred).

bufadienolide any of various naturally occurring doubly unsaturated lactones of certain steroids with important pharmacological effects on heart muscle (*see* **cardiac glycoside**). They are so named because they were originally found in the venomous secretion of the skin glands of some toads (*Bufo*), and are hence also known as **toad poisons**. They also occur in certain plants, e.g. *Digitalis*. *See also* **bufogenin B**.

buffer 1 any substance or mixture of substances that, when dissolved (usually in water), will maintain its solution at approximately constant pH despite small additions of acid or base. The commonest examples are moderately strong solutions containing both a weak acid and its conjugate base (or a weak base and its conjugate acid). A substance is useful as a buffer over a range of about one pH unit either side of its pK, but is most effective at or near the pK. Buffer substances used for biochemical or biological purposes include: acetate, bicarbonate, bis-tris propane, borate, citrate, dimethylmalonate, glycinamide, glycylglycine, imidazole, phosphate, succinate, and **Tris** together with any **Good buffer substances**. By extension, the term may be applied to agents controlling the activities of various other specified entities, e.g. redox buffer, carbon-dioxide buffer, metal-ion buffer. Also used attributively: e.g. buffer action; buffer salt; buffer solution. 2 a solution of a **buffer** (def. 1). 3 a short-term storage facility (e.g. as part of the memory of a computer), especially one whose patterns or rates of input and output can differ. 4 to treat with or to incorporate a buffer (def. 1); to act as a buffer. *See also* **buffering capacity**, **buffer value**. —**buffered** *adj.*; **buffering** *n.*

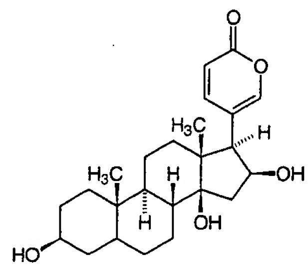
buffering capacity *or* **buffering power** 1 the number of gram-equivalents of either hydrogen ions or hydroxide ions required to change the pH of 1 litre of 1 M buffer solution by one unit. Buffering capacity = $(1/m)$ $(dn/d\text{pH})$, where m is the number of moles of buffer, and dpH is the pH change produced by addition of dn equivalents of hydrogen ions or hydroxide ions. 2 *an alternative term for* **buffer value**.

buffer solution *see* **buffer** (def. 2).

buffer value *or* **Van Slyke buffer value** *or* **buffering capacity** *symbol*: β; the amount of acid or base, in gram-equivalents, needed to change the pH of 1 litre of a buffer solution by one unit at any pH; i.e. $\beta = db/d\text{pH}$, where b is the molar concentration of base in the solution. [After Donald Dexter Van Slyke (1883–1971), US biochemist, who described it in 1922.]

buffy coat the layer of white cells that forms between the layer of red cells and the plasma when unclotted blood is centrifuged or allowed to stand.

bufogenin B 3β,14,16β-trihydroxy-5β-bufa-20,22-dienolide; a **bufadienolide** found in the Chinese drug Ch'an Su, prepared from Chinese toads (*Bufo asiaticus*).



bulge loop a structure in a polynucleotide duplex in which one strand contains a nonterminal extra sequence that is not able to base-pair with the second strand, thereby forming a bulge on one side of the duplex.

BUN *abbr. for* blood urea nitrogen, an index of the blood-urea concentration.

bundle sheath a parenchymal sheath surrounding a vascular bundle in plants.

bungarotoxin any of various neurotoxins derived from the venom of the elapid snake, *Bungarus multicinctus*. The chief components are: (1) α bungarotoxin, a single polypeptide chain of 74 amino-acid residues; it is an irreversible antagonist of nicotinic cholinergic receptors and causes paralysis; (2) β bungarotoxin, a multicomponent protein composed of two polypeptide chains: a long chain (120 amino-acid residues) and a short chain (60 residues); it prevents the release of acetylcholine from cholinergic neurons.

Bunsen coefficient *symbol*: α; the absorption coefficient of a gas in a solution. It is defined as the volume of gas in litres, reduced to 273.15 K and 1 atm (101 325 Pa) pressure, that dissolves in 1 litre of liquid when the partial pressure of the gas in the gas phase is 1 atm. [After Robert Wilhelm Bunsen (1811–99), German chemist.]

bunyavirus any of a group of RNA animal viruses consisting of enveloped particles, 90–100 nm in diameter with helical nucleocapsids, and containing segmented RNA (minus strand) of 3–4 MDa. The group has been recognized as different from **togaviruses** and includes arthropod-borne viruses that can cause encephalitis.

buoyant density the density of a solute molecule as determined by density-gradient ultracentrifugation. It is the density of the solution, ρ, at the point in the gradient where: $\rho = 1/\gamma$, where γ is the **partial specific volume** of the solute in question.

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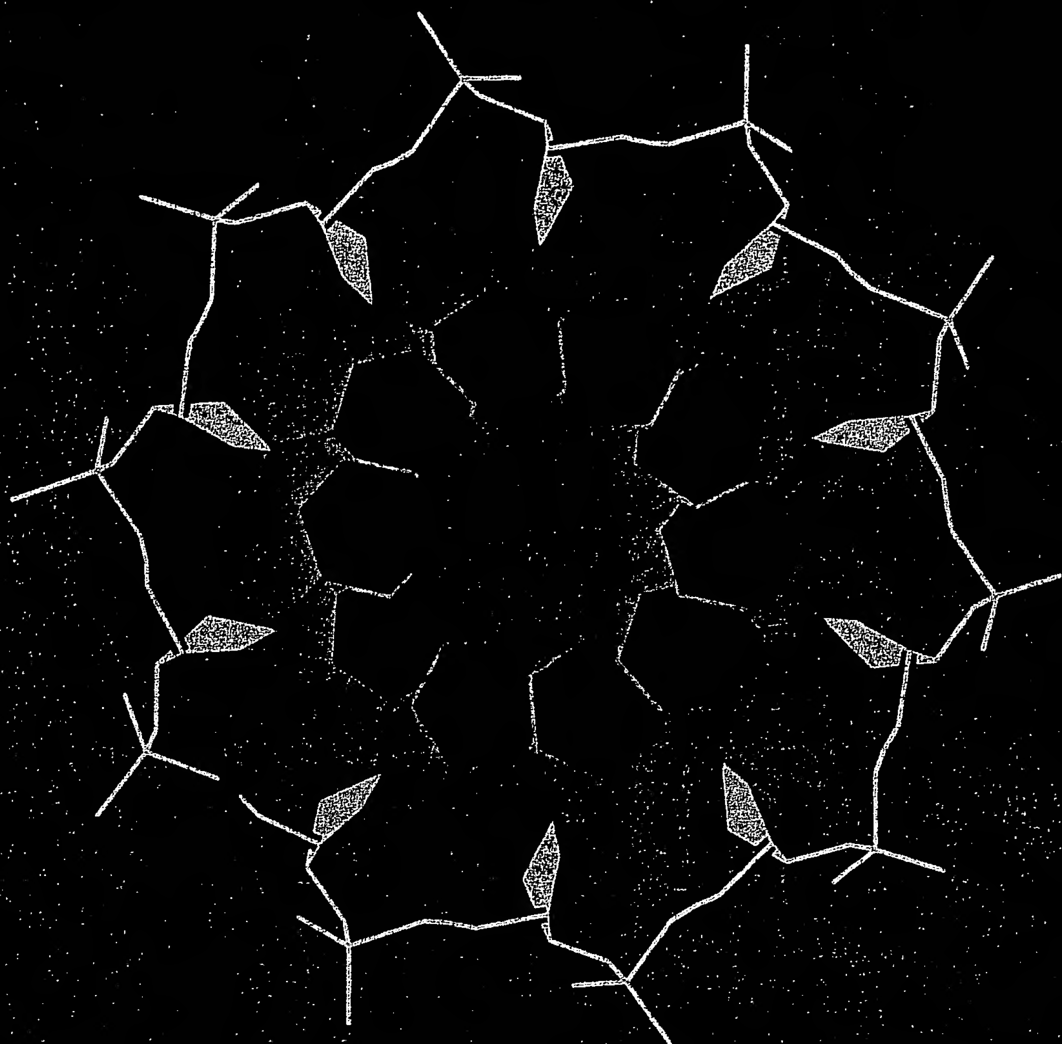

BIOCHEMISTRY

THIRD EDITION

Library of Congress Cataloging-in-Publication Data

Stryer, Lubert.
Biochemistry.

Includes index.
1. Biochemistry. I. Title.
QP514.2.S66 1988 574.19'2 87-36486
ISBN 0-7167-1843-X
ISBN 0-7167-1920-7 (international student ed.)

Printed in the United States of America

4 5 6 7 8 9 0 RRD 6 5 4 3 2 1 0 8 9

PROBLEMS

1. Tropomyosin, a 70-kd muscle protein, is a two-stranded α-helical coiled coil. What is the length of the molecule?

2. Poly-L-leucine in an organic solvent such as dioxane is α-helical, whereas poly-L-isoleucine is not. Why do these amino acids with the same number and kinds of atoms have different helix-forming tendencies?

3. A mutation that changes an alanine residue in the interior of a protein to a valine is found to lead to a loss of activity. However, activity is regained when a second mutation at a different position changes an isoleucine residue to a glycine. How might this second mutation lead to a restoration of activity?

4. An enzyme that catalyzes disulfide-sulfhydryl exchange reactions has been isolated. Inactive scrambled ribonuclease is rapidly converted into enzymatically active ribonuclease by this enzyme. In contrast, insulin is rapidly inactivated by this enzyme. What does this important observation imply about the relation between the amino acid sequence of insulin and its three-dimensional structure?

5. A protease is an enzyme that catalyzes the hydrolysis of peptide bonds of target proteins. How might a protease bind a target protein so that its main chain becomes fully extended in the vicinity of the vulnerable peptide bond?

APPENDIX
Acid-Base Concepts

I nization of Water

Water dissociates into hydronium (H_3O^+) and hydroxyl (OH^-) ions. For simplicity, we refer to the hydronium ion as a hydrogen ion (H^+) and write the equilibrium as

$$H_2O \rightleftharpoons H^+ + OH^-$$

The equilibrium constant K_{eq} of this dissociation is given by

$$K_{eq} = \frac{[H^+][OH^-]}{[H_2O]} \tag{1}$$

in which the terms in brackets denote molar concentrations. Because the concentration of water (55.5 M) is changed little by ionization, expression 1 can be simplified to give

$$K_w = [H^+][OH^-] \tag{2}$$

in which K_w is the ion product of water. At 25°C, K_w is 1.0×10^{-14}.

Note that the concentrations of H^+ and OH^- are reciprocally related. If the concentration of H^+ is high, then the concentration of OH^- must be low, and vice versa. For example, if $[H^+] = 10^{-2}$ M, then $[OH^-] = 10^{-12}$ M.

Definition of Acid and Base

An acid is a proton donor. A base is a proton acceptor.

$$\text{Acid} \rightleftharpoons H^+ + \text{base}$$

$$\underset{\text{Acetic acid}}{CH_3\text{—}COOH} \rightleftharpoons H^+ + \underset{\text{Acetate}}{CH_3\text{—}COO^-}$$

$$\underset{\text{Ammonium ion}}{NH_4^+} \rightleftharpoons H^+ + \underset{\text{Ammonia}}{NH_3}$$

The species formed by the ionization of an acid is its conjugate base. Conversely, protonation of a base yields its conjugate acid. Acetic acid and acetate ion are a conjugate acid-base pair.

Definition of pH and p*K*

The pH of a solution is a measure of its concentration of H^+. The pH is defined as

$$\text{pH} = \log_{10}\frac{1}{[H^+]} = -\log_{10}[H^+] \tag{3}$$

The ionization equilibrium of a weak acid is given by

$$HA \rightleftharpoons H^+ + A^-$$

The apparent equilibrium constant K for this ionization is

$$K = \frac{[H^+][A^-]}{[HA]} \tag{4}$$

The pK of an acid is defined as

$$\text{p}K = -\log K = \log\frac{1}{K} \tag{5}$$

Inspection of equation 4 shows that the pK of an acid is the pH at which it is half dissociated.

Henderson-Hasselbalch Equation

What is the relationship between pH and the ratio of acid to base? A useful expression can be derived from equation 4. Rearrangement of that equation gives

$$\frac{1}{[H^+]} = \frac{1}{K}\frac{[A^-]}{[HA]} \tag{6}$$

Taking the logarithm of both sides of equation 6 gives

$$\log \frac{1}{[\mathrm{H}^+]} = \log \frac{1}{K} + \log \frac{[\mathrm{A}^-]}{[\mathrm{HA}]} \qquad (7)$$

Substituting pH for $\log 1/[\mathrm{H}^+]$ and pK for $\log 1/K$ in equation 7 yields

$$\mathrm{pH} = \mathrm{p}K + \log \frac{[\mathrm{A}^-]}{[\mathrm{HA}]} \qquad (8)$$

which is commonly known as the Henderson-Hasselbalch equation.

The pH of a solution can be calculated from equation 8 if the molar proportion of A^- to HA and the pK of HA are known. Consider a solution of 0.1 M acetic acid and 0.2 M acetate ion. The pK of acetic acid is 4.8. Hence, the pH of the solution is given by

$$\mathrm{pH} = 4.8 + \log \frac{0.2}{0.1} = 4.8 + \log 2$$
$$= 4.8 + 0.3 = 5.1$$

Conversely, the pK of an acid can be calculated if the molar proportion of A^- to HA and the pH of the solution are known.

Buff ring Power

An acid-base conjugate pair (such as acetic acid and acetate ion) has an important property: it resists changes in the pH of a solution. In other words, it acts as a *buffer*. Consider the addition of OH^- to a solution of acetic acid (HA):

$$\mathrm{HA} + \mathrm{OH}^- \rightleftharpoons \mathrm{A}^- + \mathrm{H_2O}$$

A plot of the dependence of the pH of this solution on the amount of OH^- added is called a *titration curve* (Figure 2-55). Note that there is an inflection point in the curve at pH 4.8, which is the pK of acetic acid.

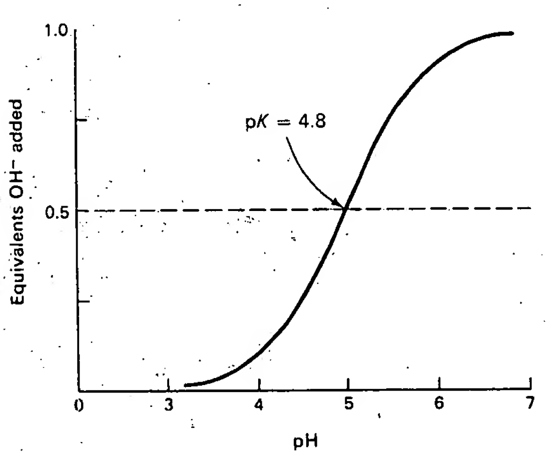

Figure 2-55
Titration curve of acetic acid.

In the vicinity of this pH, a relatively large amount of OH^- produces little change in pH. In general, a weak acid is most effective in buffering against pH changes in the vicinity of its pK value.

p*K* Values of Amino Acids

An amino acid such as glycine contains two ionizable groups: an α-carboxyl group and a protonated α-amino group. As base is added, these two groups are titrated (Figure 2-56). The pK of the α-COOH group is 2.3, whereas that of the α-$\mathrm{NH_3}^+$ group is 9.6. The pK values of these groups in other amino acids are similar. Some amino acids, such as aspartic acid, also contain an ionizable side chain. The pK values of ionizable side chains in amino acids range from 3.9 (aspartic acid) to 12.5 (arginine).

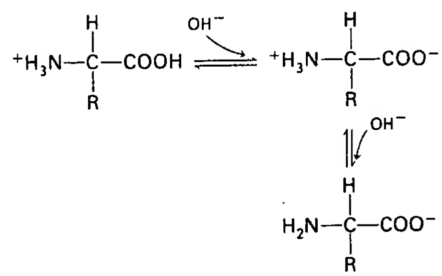

Figure 2-56
Titration of the α-carboxyl and α-amino groups of an amino acid.

Table 2-4
pK values of some amino acids

Amino acid	*pK values (25°C)*		
	α-COOH group	*α-NH_3^+ group*	*Side chain*
Alanine	2.3	9.9	
Glycine	2.4	9.8	
Phenylalanine	1.8	9.1	
Serine	2.1	9.2	
Valine	2.3	9.6	
Aspartic acid	2.0	10.0	3.9
Glutamic acid	2.2	9.7	4.3
Histidine	1.8	9.2	6.0
Cysteine	1.8	10.8	8.3
Tyrosine	2.2	9.1	10.9
Lysine	2.2	9.2	10.8
Arginine	1.8	9.0	12.5

Source: After J. T. Edsall and J. Wyman. *Biophysical Chemistry* (Academic Press, 1958), ch. 8.